

## RESEARCH ARTICLE

# Prostate-Specific Antigen Levels in Relation to Background Factors: Are there Links to Endocrine Disrupting Chemicals and AhR Expression?

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### Abstract

**Background:** Prostate-specific antigen (PSA) is a potential biomarker for early detection of prostate cancer (PCa) but its level is known to be affected by many background factors and roles of ubiquitous toxicants have not been determined. Endocrine disrupting chemicals (EDCs) are ubiquitous reproductive toxicants used in consumer products, which promote tumor formation in some reproductive model systems by binding to AhR, but human data on its expression in prostate cancer as well as its association with PSA levels are not clear. This study aimed to evaluate the expression levels of AhR and its association with serological levels of PSA and to detect possible effects of background factors and EDC exposure history on PSA levels in PCa cases. **Materials and Methods:** A cross-sectional study was conducted on the tissue levels of AhR and serum levels of PSA in 53 PCa cases from 2008-2011 and associations between each and background and lifestyle related factors were determined. **Results:** Although the AhR was overexpressed in PCa and correlated with the age of patients, it did not correlate with PSA levels. Of nutritional factors, increased intake of polysaturated fats and fish in the routine regimen of PCa cases increased the PSA levels significantly. **Conclusions:** AhR overexpression in PCa points to roles of EDCs in PCa but without any direct association with PSA levels. However, PSA levels are affected by exposure to possible toxicants in foods which need to be assessed as possible risk factors of PCa in future studies.

**Keywords:** Prostate cancer - PCa - PSA - endocrine disrupting chemicals - EDCs - AhR - environmental factors

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### Introduction

Prostate Cancer (PCa) is one of the most prevalent malignancies worldwide with a 16% lifetime risk. It is also considered as the second leading cause of cancer death in men in many parts of the world (Obort et al., 2013). While the recent incidence rates are markedly higher than rates observed three decades ago and some lifestyle and occupational factors could be considered as strong predictors of the occurrence of prostate cancer (Subahir, 2009), the most recent statistics show that prostate cancer incidence rates have now been stabilized which is thought to reflect changes in utilization of prostate-specific antigen (PSA) testing as a potential biomarker of early detection of PCa (Wright and Long, 2007), Utility of Digital Rectal Examination, Serum Prostate Specific Antigen, and Transrectal Ultrasound (Kash, 2014).

Although the only widely accepted screening tool for PCa are prostate-specific antigen (PSA) and digital rectal examination, PSA is considered to be prostate specific, but not PCa specific, and hence lacks the sensitivity to

detect a large number of tumors, especially during the early stages (Jason and Crawford, 2011). Moreover the PSA level is known to be affected by many factors, such as therapeutic regimens, previous inflammation (benign prostate hyperplasia and prostatitis), nutritional and occupational factors and urologic manipulation; hence, the controversy regarding the role of environmental factors on the incidence of PCa, (Segev and Nativ, 2006; van Weerden and Schroder, 2008; Kaba, 2014) as well as the possible role of environmental factors on PSA levels (Prins, 2008) and background factors (Ozbek, 2014) has been remained inconclusive.

Epidemiological evidence suggests that environmental factors, such as diet (Ho et al., 2011), occupation (Koifman et al., 2002; Karimi et al., 2012), metrological parameters (St-Hilaire et al., 2010) and other lifestyle factors may play significant roles on the development and progression of PCa. Out of these wide range of factors a great deal of interest is being directed towards the supposition that excessive exposure to endocrine disruptors or modulators and elevated polycyclic aromatic hydrocarbon-DNA

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adducts in benign prostate hyperplasia (Tang et al., 2013) as certain environmental chemicals may act as inducers of PCa (Golden et al., 1998; Prins et al., 2007; Medjakovic et al., 2013). Their effects may be due to mimicking the endogenous hormones such as estrogens and androgens, antagonizing their effects, changing their pattern of synthesis and metabolism and modifying the hormone receptors levels (Muir, 2005). As hormonal influences are likely related to prostate cancer etiology, hormone-modulating exogenous chemicals and their effects on PSA levels could have a particular interest which has been considered in present study.

Exposure to a wide range of chemicals via diet and other lifestyle factors may have close association with endocrine-disrupting effects including some metals (cadmium, lead, mercury, aluminum), phenolic derivatives (phenol, bisphenol-A, pentachlorophenol, resorcinol, PCBs), phthalates (used as plasticizers), variously substituted benzenes (polycyclic aromatic hydrocarbons [PAH], benzo[a]pyrene), styrenes (used in the manufacture of plastics and rubber), carbon disulphide (used in the production of rayon), dioxin, and several organochlorine pesticides, fungicides and herbicides (Pratt et al., 2011). Extremely low exposures to some endocrine modulators (plasticizers, alkylphenols) have been found to induce adverse effects on the male reproductive tract of rodents (Nilsson, 2000). Strong evidences show the Aryl hydrocarbon Receptor (AhR) mediates the toxicity of endocrine disruptors especially those with xenoestrogenic activities (Van der et al., 2009). We have investigated the roles of endocrine disruptors with xenoestrogenic activities in the incidence of benign and malignant breast tumors (Bidgoli et al., 2011) and showed how they increase the risks in premenopausal breast cancer in comparison to post menopausal breast cancer. We aimed in present study to evaluate the expression levels of AhR to find its possible association with serological levels of PSA. As the second goal we tried to detect the possible effect of background factors, including metrological, nutritional, occupational and other lifestyle factors on PSA levels in the incidence of PCa to resolve the existing questions on the role of environmental factors on the PSA levels in Iranian PCa patients.

## Materials and Methods

### *Study subject*

A case-control study was conducted on 53 prostate cancer patients and 173 normal women as control group. Both evaluated groups were randomly selected from different cities of Iran including Tehran, Shiraz, Arak, Anzali etc. We selected cases from patients who underwent surgery from 2008 to 2011 in Imam Khomeini University Hospital complex. Cases were all identified from confirmed pathological reports. The pathological feature of cases was collected from pathological reports in the pathology archive of the mentioned hospital. These patients had determined demographic information including age at diagnosis pathological features including tumor size, grade, stage, invasive state and PSA levels at the time of surgery. Other information including

reproductive history, occupational history, any familial history of cancer, smoking and alcohol consumption were collected by filling out the questionnaires by an interview with accessible patients.

### *Questionnaires*

All information was collected by two experts who were blinded to the serological levels of PSA. All variables were recorded and utilized according to standardized questionnaires/protocols that have been previously developed and successfully used by us (Bidgoli et al., 2012). Other than metrological factors (weight, height, and calculated body mass index), occupational history, physical activity (time spent in mild, moderate, and vigorous activities), daily intake of pharmaceuticals and all dietary resources of endocrine disrupting chemicals were collected.

### *Identification of environmental resources of AhR ligands*

Exact living and working addresses of cases and controls were recorded and matched with the map of factories generating PAHs and dioxins. A complete list of factories that release toxicants with hormone-like effects was made before starting the study. The men who lived within 4 km from the pollutant factories were considered as high risk people.

### *Immunohistochemical studies*

All 53 cases whose paraffinized blocks contained adjacent normal tissues were evaluated for pathological and immunohistochemical studies. As previously described (Bidgoli et al., 2011) dewaxed and rehydrated tissue sections were subjected to antigen retrieval using microwave oven and boiling citrate buffer (pH=6.0). Endogenous peroxidase activity and nonspecific binding sites were blocked by incubating sections with 0.3% hydrogen peroxide in methanol for 30 min. and 3% bovine serum albumin for 60 min, successively. Sections were then incubated 30 min at room temperature with AhR mouse monoclonal antibody (clone PRT9, abcam) that recognizes the cytoplasmic expression of human AhR in prostate tissues. The results were visualized using the envision system (Dakocytomation) based on the manufacturer's instruction with necessary modifications. Sections were also counterstained with Meyer's haematoxyline. The ideal staining conditions were established in our preliminary experiments. Method of scoring has been recently described (Arbabi Bidgoli et al., 2011).

### *Statistical methods*

Values were expressed as percent per population or as the mean±standard deviation. To assess associations between expression of AhR, serum levels of PSA and clinicopathological data, the t-student test and the nonparametric chi square test were used by SPSS 21.

## Results

### *AhR levels in prostate cancer patients*

Out of 53 patients whose tissues were stained by IHC

methods, AhR overexpression strong(4+) staining was recorded in 45 (83%) of epithelial cells of prostate cancer patients whereas it was found in 28(58%) of adjacent stromal cells of malignant tumors (Figure 1A) when compared with adjacent normal tissues.

The AhR levels were showed a close inverse association with the age of patients that means younger patients showed lower levels of AhR staining in their epithelial cells (70.3±3.8 vs 65. 2±4.6 yrs, p<001). No statistical association was found between tissue levels of AhR and serum levels of PSA in evaluated PCa patients.

#### Clinicopathological importance of PSA levels in PCa patients

PSA levels were not associated with pathological features, age, height, weight, BMI, physical activities and many other lifestyle factors of patients(data was not

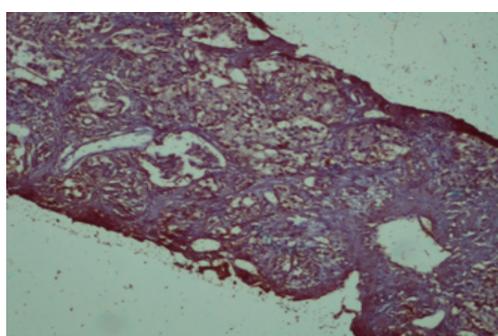


Figure 1. Strong (4+) AhR Expression in Epithelial and Stromal Cells of Prostate Cancer [(x100) and (x400)]

Table 1. Role of Some Nutritional Factors on the Level of PSA in PCa Patients(n=53)

Characteristics	PSA (mean±SD) levels	p value
Saturated oil	33.38±8.4	0.01*
Polyunsaturated vegetable	27.30±3.5	
Monounsaturated oil	9±2.8	
Egg	4/day 19.45±4.73	0.08
<4/day	27.86±5.53	
Fish	No 18.50±3.6	0.001*
1/week	25.47±4.3	
2/week	39.18±3.8	
Omega3 supplement	Yes 11.67±2.9	0.08
No	27.92±3.3	
Soy products	Yes 26.68±3.8	0.9
No	27.20±3.65	
Other dietary phytoestrogens	1-2time/week 19.07±5.4	0.34
3-5time/week	28.22±3.8	
≥5time/week	28.10±4.6	
Lycopene	1-2 times/week 33.40±2.9	0.61
3-5times/week	26.54±4.3	
5-7times/week	25.71±3.5	
Sugar consumption	Yes 27.02±2.9	0.98
No	26.2±4.3	
Fatty	Yes 25.49±3.8	0.06
No	36.93±3.4	

Table 2. Role of AhR Agonists and Antagonists on the Level of PSA in PCa Patients(n=53)

Characteristics	PSA levels ng/ml (mean±SD)	p value	
Smoking	No	26.20±3/9	0.17
	Experimental	38.63± 4.6	
	Recreational	30.77±3.3	
	Habitual	25.67±5.3	
	Abuse	17.94±5.9	
Roasted meat	No	30.25±4.7	0.36
	1time/week	28.05±3.5	
	2times/week	20.00±4.5	
Grilled meat	No	28.27± 5.4	0.77
	1time/week	30.19±6.9	
	2times/week	24.39±8.4	
	3times/week	24.00±5.4	
Occupational Exposure to PAHs	Yes	26.58±4.3	0.88
	No	27.24±5.7	
Living near PAHs producing factories (<4km)	Yes	19.83±3.4	0.24
	No	27.91±5.8	
Using plastic dishes and bottles	Yes	26.85±5.4	0.94
	No	27.15±4.8	
Lemon	Yes	25.85±3.7	0.46
	No	29.05±5.3	
Green tea	Yes	29.42±4.9	0.53
	No	26.29±5.8	
Nuts	Yes	27.73±3.1	0.63
	No	25.58±4.3	
Grape	Yes	27.98±4.1	0.24
	No	20.58±3.8	

showed). Its level didn't affect also by many background factors e. g. familial history of any malignancy, history of background diseases, type of pharmaceutical regimens and some of nutritional factors but surprisingly its level was affected by routine intake of some foods (Table 1).

#### Role of environmental agonists and antagonists on PSA levels

The direct role of exposure to AhR agonists and antagonists on the PSA levels was described in table 2. As recognized AhR inducers smoking, grilled meat, high temperature processed meat, and living near or working in PAHs producing factories were considered without any significant association with PSA levels. On the other side AhR antagonists including lemon, grape, nuts and green tea were considered without any contribution.

#### Discussion

When we started this study there was an increasing concern regarding the impact of Endocrine disrupting chemicals (EDCs) on prostate cancer development and progression (Cillo et al., 2007). One of the major group of EDCs are carcinogenic polycyclic aromatic hydrocarbons (PAHs) which are known as genotoxic agents and ligands of the AhR. AhR has been suggested to have an important role in prostate carcinogenesis (Hrubal et al., 2011) and environmental and developmental exposure to EDCs

especially PAHs with estrogenic activity induce estrogen reprogramming of the prostate gland and causes permanent alterations in structure and gene expression which may lead to an increased risk of prostate cancer with aging (Hu et al., 2012). EDCs act directly through interaction with estrogen receptors (ERs) or indirectly through activation of AhR or by modulation of critical metabolic enzymes engaged in estrogen biosynthesis and metabolism (Schmidt and Peterlin-Masic 2012). We aimed in the present study to evaluate the expression levels of AhR in prostate cancer tissues for the first time and we showed how this receptor with its critical roles in cancer progression overexpressed in malignant cells of 45 cases (85%) and stromal cell of 28 cases (53%) in comparison to adjacent normal tissues as the first important achievement of this study. As the increased incidence of prostate cancer has been reported in men exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which acts through the AhR and interacts with the androgen receptor (AR) (Bjork et al., 2013) therefore the association between tissue levels of AhR and serum levels of Dioxins is highly suggested for future studies in different exposed population as partially considered before without any concern to AhR levels (Hu et al., 2010).

We detected also a close association between AhR levels and age of PCa patients because older patients showed higher levels of AhR staining in their epithelial cells (70.3±3.8 vs 65.2±4.6 yrs,  $p < 0.001$ ). The estrogen reprogramming of the prostate gland is a function of developmental exposures (aka developmental estrogenization) which develops with aging (Schmidt and Peterlin-Masic, 2012) and AhR levels in older men reflected this fact in our patients clearly. Since it has been suggested that stem cells and cancer stem cells are potential targets of cancer initiation and disease management, it is highly possible that estrogens and EDCs influence the development and progression of prostate cancer through reprogramming and transforming the prostate stem and early stage progenitor cells therefore the AhR roles in these two cell types should be considered in future studies as a prostate cancer prevention target.

As hormone-modulated effects of exogenous chemicals hormonal are likely related to prostate cancer etiology by AhR activation we looked for its possible association on PSA levels in present study as the second new goal of our study. Although recent work didn't show any association between these two important parameters, their contribution in early development of prostate cancer even in precancerous lesions and Benign Prostate Hyperplasia (BPH) should be considered in next studies certainly. At the next step we looked for possible association between PSA levels and exposure to AhR agonists e. g. smoking (Anderson et al., 2014), PAHs contained cooked food e. g. grilled and roasted meats, occupational exposure to PAHs and living near PAHs producing factories with less 4 km distance and in this assessment no clear association was detectable between these PAHs exposing lifestyle factors and PSA levels. The direct role of PAHs on the incidence of PCa and BPH in Iranian population are discussing issues in our parallel works. At the same time consumption of AhR antagonists including grape, lemon, green tea and nuts didn't show

any effect on PSA levels.

Out of nutritional factors increased intake of polysaturated fats and fish in the routine regimen of PCa increased the PSA levels significantly but their specific roles in the incidence of PCa should be checked later. AhR ligands are potent toxicants which are widespread released to the environment and resistant to metabolic breakdown. The latter factor is responsible for the accumulation of these compounds in the food chain therefore their EDCs levels, PAHs levels especially the xenoestrogenic properties of these foods should be assessed and their sustained effects on prostate cancer should be assessed in next studies.

In conclusion, although we showed clearly the role of EDCs as AhR inducers in Iranian PCa patients in an age dependent manner, it didn't show any association with PSA levels. Moreover PSA in this well grown tumors was not associated with background factors and history of PAHs exposure but the situation should be checked in benign and early stage tumors. Some significant effects of special nutritional factors on PSA levels especially fish and saturated oils suggests the importance of diet in prevention and control of PCa which needs specific concerns on the role of nutritional factors on the incidence of PCa as an attractive subject for future studies.

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## References

- Anderson RA, McIlwain L, Coutts S, et al (2014). Activation of the aryl hydrocarbon receptor by a component of cigarette smoke reduces germ cell proliferation in the human fetal ovary. *Mol Hum Reprod*, **1**, 42-8.
- Bidgoli SA, Eftekhari T, Sadeghipour R (2011). Role of xenoestrogens and endogenous sources of estrogens on the occurrence of premenopausal breast cancer in Iran. *Asian Pac J Cancer Prev*, **12**, 2425-30.
- Bidgoli SA, Khorasani H, Keihan H, Sadeghipour A, Mehdizadeh A (2012). Role of endocrine disrupting chemicals in the occurrence of benign uterine leiomyomata: special emphasis on AhR tissue levels. *Asian Pac J Cancer Prev*, **13**, 5445-50.
- Björk C, Lundberg Giwercman Y (2013). Androgen receptor CAG repeat length modifies the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on receptor activity in human prostate cells. *Reprod Toxicol*, **35**, 144-9.
- Cillo F, de Eguileor M, Gandolfi F, Brevini TA (2007). Aroclor-1254 affects mRNA polyadenylation, translational activation, cell morphology, and DNA integrity of rat primary prostate cells. *Endocr Relat Cancer*, **14**, 257-66.
- Golden RJ, Noller KL, Titus-Ernstoff L, et al (1998). Environmental endocrine modulators and human health: an assessment of the biological evidence. *Crit Rev Toxicol*, **280**, 109-227.
- Ho E, Beaver LM, Williams DE, Dashwood RH (2011). Dietary Factors and Epigenetic Regulation for Prostate Cancer Prevention. *Adv Nutr*, **2**, 497-510.
- Hruby E, Vondracek J, Libalova H, et al (2011). Gene expression changes in human prostate carcinoma cells exposed to genotoxic and nongenotoxic aryl hydrocarbon receptor

- ligands. *Toxicol Lett*, **206**, 177-88.
- Hu WY, Shi GB, Hu DP, Nelles JL, Prins GS (2012). Actions of estrogens and endocrine disrupting chemicals on human prostate stem/progenitor cells and prostate cancer risk. *Mol Cell Endocrinol*, **354**, 63-73.
- Hu WY, Shi GB, Hu DP, Nelles JL, Prins GS ((2010)). Associations of serum concentrations of organochlorine pesticides with breast cancer and prostate cancer in U. S. adults. *Environ Health Perspect*, **118**, 60-6.
- Jason MP, Crawford ED (2011). Prostate-specific antigen (PSA) screening: has the pendulum swung too far? *AJA*, **13**, 655-6.
- Kaba M, Pirincci N, Yuksel MB, et al (2014). Serum levels of trace elements in patients with prostate cancer. *Asian Pac J Cancer Prev*, **15**, 2625-9.
- Karimi G, Shahar S, Homayouni N, et al (2012). Association between Trace Element and Heavy Metal Levels in Hair and Nail with Prostate Cancer. *Asian Pac J Cancer Prev*, **13**, 4249-53.
- Kash DP, Lal M, Hashmi AH, Mubarak M (2014). Utility of digital rectal examination, serum prostate specific antigen, and transrectal ultrasound in the detection of prostate cancer: a developing country perspective. *Asian Pac J Cancer Prev*, **15**, 3087-91.
- Koifman S1, Koifman RJ, Meyer A (2002). Human reproductive system disturbances and pesticide exposure in Brazil. *Cad Saude Publica*, **18**, 435-45.
- Medjakovic, Zoechling A, Gerster P, et al (2013). Effect of nonpersistent pesticides on estrogen receptor, androgen receptor, and aryl hydrocarbon receptor. *Environ Toxicol*.
- Muir KR (2005). Endocrine-disrupting pesticides and selected hormonally dependent cancers. *Scand J Work Environ Health*, **31**, 55-61.
- Nilsson (2000). Endocrine modulators in the food chain and environment. *Toxicol Pathol*, **28**, 420-31.
- Obort AS, Ajadi MB, Akinloye O (2013). Prostate-Specific Antigen: Any Successor in Sight? *Rev Urol*, **15**, 97-107.
- Ozbek E, Otunctemur A, Dursun M, et al (2014). Diabetes Mellitus and HbA1c Levels Associated with High Grade Prostate Cancer. *Asian Pac J Cancer Prev*, **15**, 2555-8.
- Pratt MM, Kaarthik John, Allan B. MacLean, Senait Afework, David H. Phillips, Miriam C (2011). Poirier. Polycyclic Aromatic Hydrocarbon (PAH) Exposure and DNA Adduct Semi-Quantitation in Archived Human Tissues. *Int J Environ Res Public Health*, **8**, 2675-91.
- Prins GS, Birch L, Tang WY, Ho SM (2007). Developmental estrogen exposures predispose to prostate carcinogenesis with aging. *Reprod Toxicol*, **23**, 374-82.
- Prins GS (2008). Endocrine disruptors and prostate cancer risk. *Endocr Relat Cancer*, **15**, 1.
- Schmidt J, Peterlin-Masic L (2012). Organic synthetic environmental endocrine disruptors: structural classes and metabolic fate. *Acta Chim Slov*, **59**, 722-38.
- Segev Y, Nativ O (2006). Nutrition and pharmacological treatment for prevention of prostate cancer. *Harefuah*, **145**, 76-7.
- St-Hilaire S, Mannel S, Commendador A, Mandal R, Derryberry D (2010). Correlations between meteorological parameters and prostate cancer. *Int J Health Geogr*, **21**, 1-11.
- Subahir MN, Shah SA, Zainuddin ZM (2009). Risk factors for prostate cancer in Universiti Kebangsaan Malaysia Medical Centre: a case-control study. *Asian Pac J Cancer Prev*, **10**, 1015-20.
- Tang D, Kryvenko ON, Wang Y, et al (2013). Elevated polycyclic aromatic hydrocarbon-DNA adducts in benign prostate and risk of prostate cancer in African Americans. *Carcinogenesis*, **34**, 113-20.
- Van der HE, Bechoux N, Muller M et al. Food flavonoid aryl hydrocarbon receptor-mediated agonistic/antagonistic/synergic activities in human and rat reporter gene assays. *Anal Chim Acta*, **637**, 337-45.
- van Weerden WM, Schroder FH (2008). The use of PSA as biomarker in nutritional intervention studies of prostate cancer. *Chem Biol Interact*, **17**, 204-11.
- Wright JL, Lange PH (2007). Newer potential biomarkers in prostate cancer. *Rev Urol*, **9**, 207-13.